

sumed by predators who leave the empty shells. However, females have heavier soft parts than males (GUEDES *et al.*, 1981). Because the intercepts of the biomass regressions of both sexes are significantly different, BOURNE & BERLIN (1982) stated that "an overall equation, although possibly still useable, would show a consistent bias in prediction depending on the sex of the individual." We suggest that it is possible to identify reliably the sex of each specimen and to use differentiated regression equations to predict the biomass.

GUEDES *et al.* (1981) defined an operculum product (length \times width) that they used as an independent variable in a regression on living weight. This parameter seems to be inadequate, considering the observed sexual differences in operculum width. A female will have a lower operculum product but a greater biomass than a male of the same operculum length, and the dispersion from an overall regression curve will therefore be greater.

The identification of sex in empty shells should make it possible to establish whether predation by kites and limpkins exerts the same pressure on both sexes, since random capture on the normally 1:1 sex ratio of *Pomacea* populations (MARTIN, 1984)³ would yield an equivalent ratio in the mounds of shells at feeding sites. Should this not be the case, then differential activities in either sex—as have already been found in other prosobranchs (RIBI & ARTER, 1986)—and/or other factors affecting their capturability should be included in the analysis.

Taxonomy: Some detailed studies on the anatomy of several species are available, but most ampullariids have still not been well-defined (PAIN, 1972). Though shells are variable, the form of the aperture has frequently been used as a diagnostic character. At least in two genera, some taxonomic doubts can be dispelled through an analysis of sexual dimorphism in the shell.

PILSBRY (1933) described *Marisa planogyra*, from Brazil, as differing from the type species, *M. cornuarietis*, based on, among other things, the fact that "the last whorl and the aperture are less expanded in the upper and basal parts" in the former. Taking into account the results of HALE (1964) and DEMIAN & IBRAHIM (1972), as well as our present findings, this diagnostic characteristic of *M. planogyra* could correspond to female shells. OLAZARRI (1977) suggested that the two species could be synonyms.

Also, a significant variation in the aperture was mentioned in the supraspecies *Pomacea canaliculata* (*sensu* CAZZANIGA, 1987). For example, *P. levior* (Sowerby, 1909) was differentiated by its expanded outer lip. GEIJSKES & PAIN (1957), studying *P. dolioidea* (Reeve, 1854) from Surinam, found that "in some specimens the peristome is wider than normally as in those described by him [Vernhout, 1914] as *levior*." A similar observation was also made

by PAIN (1960) with regard to *P. lineata* (Spix in Wagner, 1827) from Brazil, in whose synonymy he placed *P. levior*. In both cases the specimens appear to have been male.

A series of similar cases is easily found in the already confused scheme of *Pomacea* forms. To what extent are many of the mentioned differences among nominal species due to an unnoticed sex dimorphism? Knowledge of many South American species of *Pomacea* is based on nothing more than a few empty shells, without any assessment of variability. A test for the presence of a dimorphic condition in the other apple snails would improve the understanding of the conchological species: several putative species could prove to be sex-based synonyms.

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³ It should be noted that the sex ratio balance was tipped in favor of the females in the populations of *Marisa cornuarietis* studied by DEMIAN & IBRAHIM (1972).

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The Use of Tetracycline Staining Techniques to Determine Statolith Growth Ring Periodicity in the Tropical Loliginid Squids *Loliolus noctiluca* and *Loligo chinensis*

by

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Abstract. The tropical near-shore loliginid squids *Loliolus noctiluca* and *Loligo chinensis* have statolith growth rings similar to those described for other squid species. These rings also appear to be similar in appearance to increments found in fish otoliths. Daily periodicity of these rings was validated by staining the statoliths with tetracycline and comparing the number of rings produced with the elapsed days. These results were considered in relation to previous validation work on statolith rings that has been carried out with other squid species. Microstructural examination of statoliths promises to be a useful tool to obtain future growth information for these tropical loliginid squids.

INTRODUCTION

Growth rings within the statolith microstructure of the pelagic squids are gaining increased attention as possible chronological time marks. Currently there is a considerable lack of knowledge on the population dynamics of many squid species; basic biological information is lacking on life span, age at maturity, and growth rates. Research on statolith growth increments promises to yield a useful tool to obtain some of this vital information. However, unequivocal demonstration of periodicity in these growth rings is essential.

Statolith growth rings have been observed and counted in several squid species (HURLEY & BECK, 1979; KRISTENSEN, 1980; ROSENBERG *et al.*, 1981; NATSUKARI *et al.*, 1988) and daily ring periodicity has been documented in some (HURLEY *et al.*, 1985; LIPINSKI, 1986; JACKSON, 1989, 1990). The purpose of this study was to investigate statolith ring periodicity in two other tropical loliginids, *Loligo chinensis* (Gray, 1849) and *Loliolus noctiluca* (Lu *et al.*, 1985), using tetracycline staining techniques. *Loligo chinensis* is found throughout much of the tropical Indo-Pacific (ROPER *et al.*, 1984) while *Loliolus noctiluca* is a smaller near-shore

species that is found along the east coast of Australia from New Guinea to Tasmania (LU *et al.*, 1985).

MATERIALS AND METHODS

Loliolus noctiluca

Young individuals of *Loliolus noctiluca* were captured using 1-mm and 8-mm mesh seine nets off the beach at Townsville, North Queensland, Australia. Captured squids were transported back to the laboratory in 20-L plastic buckets and their statoliths were stained by exposing them to an ambient solution of 250 mg of tetracycline per liter of seawater as described in JACKSON (1989). Squids were captured in June and July 1989 and were maintained alive until sacrificed after 30 and 31 days, respectively, although some mortality occurred during maintenance. Squids were kept outside in a 1500-L round fiberglass tank connected to a recirculating seawater system. Three squids captured in June were transferred to a 308-L round tank on day 30 and allowed to grow until they died after 77 and 83 days. Food was supplied *ad libitum* by maintaining live sergestid shrimps *Acetes sibogae australis* (<3



Figure 1

A. Daily growth rings in a whole statolith of a field-captured *Lololus noctiluca* (female, 59 days, 52 mm dorsal mantle length) mounted in thermoplastic cement. Scale bar = 50 μ m. B. Daily growth rings in a whole statolith of a field-captured *Loligo chinensis*, which has been ground and polished on both sides to produce a thin section (male, 78 days, 110 mm dorsal mantle length). Scale bar = 100 μ m.

cm in length) with the squids. Large schools of this easily obtainable food source were maintained with *Lololus noctiluca* and used as a constant and abundant food supply.

Loligo chinensis

Individuals of *Loligo chinensis* were trawled using 40-mm mesh, paired otter trawls in Cleveland Bay off Townsville on 13 July 1989. Although individuals were often killed during trawling, any squids that were in good condition were placed immediately in a 98-L tub with flow-through seawater. Although mortality was high, there was some survival during the course of the day. Because *Loligo chinensis* is large and sensitive to handling, exposing it to an ambient solution of tetracycline-seawater was not suitable; therefore, an injection technique was used. Squids brought back from trawling were injected with a tetracycline-seawater solution (6 mg/mL) at the base of arm I. Previous injection trials indicated that tetracycline was incorporated into the statolith within at least 15 hr of injection, e.g., an individual that was injected in the evening of the day of trawling and found dead the following morning had already taken up the tetracycline into its statolith.

Two individuals of *Loligo chinensis* survived capture and injection and were maintained for 21 and 25 days, respectively, in a 2500-L circular tank that was maintained outside and equipped with a closed recirculating seawater system. Live food organisms kept with the squids were fishes of the families Ambassidae, Mugilidae, and Sillaginidae and juvenile penaeid and *Acetes* crustaceans. Feeding was *ad libitum*.

Statolith Observation

Details regarding grinding techniques, delineation of the tetracycline mark, and counting of subsequent growth rings are the same as described for *Sepioteuthis lessoniana* (JACKSON, 1990). Growth rings in the statoliths of *Lololus noctiluca* were counted directly without any grinding or polishing, as growth ring definition is excellent in statoliths that are not ground (Figure 1a). Rings were most visible in the dorsal dome region. Similarly the outermost rings could be visualized on the dorsal dome of the statolith of the larger *Loligo chinensis* without any polishing or grinding. However, the statolith of the second individual required grinding and polishing on both sides to enhance the visibility of the growth rings. This resulted in the growth rings being most easily delineated on the rostrum. To delineate all the growth rings clearly within the statolith microstructure of *Loligo chinensis* generally requires grinding and polishing of the statolith on both surfaces to produce a thin section (Figure 1b).

RESULTS AND DISCUSSION

The mean value obtained from replicate growth ring counts from the tetracycline mark to the statolith edge for both *Lololus noctiluca* (Table 1) and *Loligo chinensis* (Table 2)

Table 1

Tetracycline staining and statolith ring counts for *Loliolus noctiluca* (SD = standard deviation).

Sex	Mantle length (mm)	Date stained	Date experiment terminated	Number of days	Replicate statolith ring counts	Mean	SD
F	35.0	6 June 1989	20 June 1989	14	14, 14, 13	14	0.58
J	19.8	6 June 1989	21 June 1989	15	15, 14, 16	15	1.00
F	38.0	7 June 1989	7 July 1989	30	30, 29, 28	29	1.00
M	32.0	7 June 1989	7 July 1989	30	28, 27, 28	28	0.58
F	34.0	7 June 1989	7 July 1989	30	29, 29, 31	30	1.15
M	30.0	7 July 1989	7 August 1989	31	30, 30, 31	30	0.58
M	38.0	7 June 1989	23 August 1989	77	79, 75, 75	76	2.31
F	45.0	7 June 1989	30 August 1989	83	86, 82, 89	86	3.51
F	54.0	7 June 1989	30 August 1989	83	77, 82, 84	81	3.61

corresponded to, or was very close to, the number of days the squids were maintained. Individuals of *Loliolus noctiluca* were maintained for periods of 13 to 83 days. The degree of correspondence between the days maintained and ring number decreased and the among-count variance increased with the length of time maintained. This reflects the problems associated with counting large numbers of relatively narrow rings.

Statolith growth ring analysis promises to be the most useful method for establishing squid age. However, the technique is of value only when ring counts are highly accurate, which requires experience and familiarity with the ring structure of the species studied. For example, validation of daily rings in *Sepioteuthis lessoniana* has highlighted the presence of sub-daily rings (JACKSON, 1990), which if counted would lead to an over estimation of squid age. Because of the specificity involved in ring counting, it is often difficult to obtain independent counts from multiple observers. Avoiding observer bias is therefore important. This is most easily achieved by using a hand counter during counts so the observer is not biased by previous trials. In addition, replicate counts should be made of each statolith to provide estimates of variance in ring numbers. It would be of use in future work to establish a core of cephalopod workers with expertise and experience in statolith ring counting. In this way both the intra- and inter-observer counting biases could be addressed systematically.

The growth rings present in the squid statolith are similar to those found in fish otoliths. Having the ability to obtain accurate age estimates from fishes has proven to be

valuable to the understanding of their biology (e.g., CAMPANA & NEILSON, 1985). It is becoming increasingly apparent that squid statoliths can be used in a similar way to ascertain important biological parameters that would be difficult to obtain by other means (e.g., NATSUKARI *et al.*, 1988). However, the results obtained are only tentative until the periodicity of statolith growth rings can be calibrated.

Techniques used in the analysis of growth ring data from fish otoliths can be applied to the study of statolith microanatomy. These include delineation of growth rings using scanning electron microscopy on polished and etched statolith surfaces (RADTKE, 1983; HURLEY & BECK, 1979; LIPINSKI, 1986). Alternatively, light microscopy has been used to observe growth rings in whole untreated statoliths (BALCH *et al.*, 1988; JACKSON, 1989) or in statoliths that have been ground and polished using various techniques (KRISTENSEN, 1980; ROSENBERG *et al.*, 1981; NATSUKARI *et al.*, 1988; JACKSON, 1990).

Artificially inducing a chemical time mark on the statolith is perhaps one of the most convenient methods for the validation of daily statolith ring periodicity. Culturing squids from hatching (thereby knowing the age of individuals) is the only other means to calibrate ring periodicity (e.g., YANG *et al.*, 1986), and this method is often quite difficult and time consuming. Exposing squids to an ambient solution of tetracycline or calcein-seawater is most easily used with small individuals that can easily be maintained in relatively small confines during the staining process, with minimal damage, e.g., *Idiosiphus pygmaeus*

Table 2

Tetracycline injection and statolith ring counts for *Loligo chinensis* (SD = Standard Deviation).

Sex	Date injected	Date experiment terminated	Number of days	Replicate statolith ring counts	Mean	SD	Area of statolith observed
M	13 July 1989	7 August 1989	25	24, 26, 26, 26, 23, 24	25	1.33	Rostrum
M	13 July 1989	3 August 1989	21	20, 20, 20, 22, 21, 22	21	0.98	Dorsal dome